

We claim:

1. A method for identifying a virulence gene of *M. marinum*, comprising
 - a) mutagenizing an *M. marinum* bacterium by introducing into the bacterium a plasmid which comprises a signature-tagged transposon, whereby the transposon integrates into and disrupts a gene in the bacterium,
 - b) introducing the mutagenized bacterium into a host susceptible to infection thereof,
 - c) identifying a bacterium which comprises a signature tagged transposon and which exhibits reduced viability in the host, compared to a non-mutagenized *M. marinum* bacterium,
 - d) cloning and/or sequencing a nucleic acid sequence which flanks the integrated transposon in said identified bacterium, and
 - e) identifying a wild type *M. marinum* gene which comprises at least a portion of said flanking sequence.
2. The method of claim 1, further comprising
 - f) confirming that the mutation renders *M. marinum* less virulent.
3. A method of constructing an avirulent *M. marinum* bacterium, comprising mutagenizing an *M. marinum* virulence gene identified by the method of claim 1.
4. An avirulent *M. marinum* bacterium, produced by the method of claim 3.
5. An avirulent *M. marinum* bacterium, in which one or more genes comprising a nucleic acid of SEQ ID NOs: 4, 6, 8, 10, 11, 13, 21, 23, 25, 27, 29, 31, 35, 39, 41, 43 or 44 is mutated.
6. A method for identifying a virulence gene of *M. tuberculosis*, comprising identifying a virulence gene of *M. marinum* bacterium according to the method of claim 1, and further comprising,

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comparing said flanking nucleic acid sequence to a databank of *M. tuberculosis* nucleic acid sequences, and/or comparing the sequences of peptides which are coded for by said flanking sequences to a known *M. tuberculosis* protein database, and

5 identifying an *M. tuberculosis* gene which comprises a sequence that is substantially identical to said flanking sequences.

7. A method for generating an avirulent *M. tuberculosis* bacterium, comprising mutagenizing an *M. tuberculosis* virulence gene identified by the method of claim 6.

8. An avirulent *M. tuberculosis* bacterium, produced by the method of claim 7.

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10 9. An avirulent *M. tuberculosis* bacterium, in which one or more of genes Rv0822c, CY20G9.23 (Rv0497), the pks family, including e.g., ppsE (Rv2935), psk6 (Rv0405), pks9 (Rv1664), pks8 (Rv1662), pks1 (Rv2946c), and pks002c, Rv3511, O08381 (Rv0357c), Rv3775, Rv3137, Rv2348c, Rv3860, mbtB (Rv2383c), Rv2181, Rv1954c, Rv0987, Rv3268, Rv2610c, nrp (pir E70751, Rv0101), mbtE (Rv2380c), Rv0236c or smc (Rv2922c) is mutated to render the *M. tuberculosis* bacterium less
15 virulent.

10. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv0822c is mutated.

11. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene CY20G9.23 is mutated.

20 12. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene ppsE is mutated.

13. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene pks6 is mutated.

25 14. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene pks9 is mutated.

15. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene pks8 is mutated.

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16. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene pks1 is mutated.
17. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene pks002c is mutated.
- 5 18. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv3511 is mutated.
19. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene 008381 is mutated.
- 10 20. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv3775 is mutated.
21. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv3137 is mutated.
22. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv2348c is mutated.
- 15 23. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv3860 is mutated.
24. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene mbtB is mutated.
- 20 25. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv2181, Rv1954c, Rv0987, Rv3268, or Rv2610c is mutated.
26. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene npr (pirE 70751) or mbtE is mutated.
27. An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv0236c or smc is mutated.
- 25 28. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 4, or a variant or fragment thereof.

29. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 6, or a variant or fragment thereof.

30. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 8, or a variant or fragment thereof.

5 31. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 11, or a variant or fragment thereof.

32. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 13, or a variant or fragment thereof.

10 33. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 21, or a variant or fragment thereof.

34. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 23, or a variant or fragment thereof.

35. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 25, or a variant or fragment thereof.

15 36. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 27, or a variant or fragment thereof.

37. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 29, or a variant or fragment thereof.

20 38. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 31, or a variant or fragment thereof.

39. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 39, or a variant or fragment thereof.

40. An isolated nucleic acid comprising the oligonucleotide of SEQ ID NO: 41, or a variant or fragment thereof.

25 41. A pharmaceutical composition, comprising an avirulent *M. marinum* bacterium of claim 5 and a pharmaceutically acceptable carrier.

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42. An attenuated *M. marinum* vaccine, comprising an avirulent *M. marinum* bacterium of claim 5 and a pharmaceutically acceptable carrier.

43. A pharmaceutical composition, comprising an avirulent *M. tuberculosis* bacterium of claim 9 and a pharmaceutically acceptable carrier.

5 44. An attenuated *M. tuberculosis* vaccine, comprising an avirulent *M. tuberculosis* bacterium of claim 9 and a pharmaceutically acceptable carrier.

45. An attenuated *M. tuberculosis* vaccine, comprising an avirulent *M. tuberculosis* bacterium which comprises one or more mutations in one or more virulence genes identified by the method of claim 7 and a pharmaceutically acceptable carrier.

10 46. A method to elicit an immune response in a fish in need of such treatment, comprising administering to said fish an avirulent *M. marinum* bacterium of claim 5.

47. A method to elicit an immune response in a patient in need of such treatment, comprising administering to said patient an avirulent *M. tuberculosis* bacterium of claim 9.

15 48. An isolated polyketide made by the polyketide synthase encoded by the *M. marinum* polyketide synthase gene which comprises the oligonucleotide of SEQ ID NO:8.

49. An isolated polyketide made by the *M. tuberculosis* polyketide synthase gene ppsE, pks6, pks8, pks9, pks1 or pks002c .

20 50. A method for isolating a mutagenized *M. marinum* bacterium which exhibits reduced virulence in a host susceptible to infection thereof compared to a non-mutagenized *M. marinum* bacterium, comprising integrating a tagged transposon into the DNA of a *M. marinum* bacterium in a manner effective to produced reduced virulence, and isolating said mutagenized bacterium.

25 51. An isolated nucleic acid of claim 28, consisting essentially of the oligonucleotide of SEQ ID NO: 4, or a variant or fragment thereof.

52. An isolated nucleic acid of claim 29, consisting essentially of the oligonucleotide of SEQ ID NO: 6, or a variant or fragment thereof.

53. An isolated nucleic acid of claim 30, consisting essentially of the oligonucleotide of SEQ ID NO: 8, or a variant or fragment thereof.

54. An isolated nucleic acid of claim 31, consisting essentially of the oligonucleotide of SEQ ID NO: 11, or a variant or fragment thereof.

5 55. An isolated nucleic acid of claim 32, consisting essentially of the oligonucleotide of SEQ ID NO: 13, or a variant or fragment thereof.

56. An isolated nucleic acid of claim 33, consisting essentially of the oligonucleotide of SEQ ID NO: 21, or a variant or fragment thereof.

10 57. An isolated nucleic acid of claim 34, consisting essentially of the oligonucleotide of SEQ ID NO: 23, or a variant or fragment thereof.

58. An isolated nucleic acid of claim 35, consisting essentially of the oligonucleotide of SEQ ID NO: 25, or a variant or fragment thereof.

59. An isolated nucleic acid of claim 36, consisting essentially of the oligonucleotide of SEQ ID NO: 27, or a variant or fragment thereof.

15 60. An isolated nucleic acid of claim 37, consisting essentially of the oligonucleotide of SEQ ID NO: 29, or a variant or fragment thereof.

61. An isolated nucleic acid of claim 38, consisting essentially of the oligonucleotide of SEQ ID NO: 31, or a variant or fragment thereof.

20 62. An isolated nucleic acid of claim 39, consisting essentially of the oligonucleotide of SEQ ID NO: 39, or a variant or fragment thereof.

63. An isolated nucleic acid of claim 40, consisting essentially of the oligonucleotide of SEQ ID NO: 41, or a variant or fragment thereof.

25 64. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 51.

65. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 52.

5 66. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 53.

67. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 54.

10 68. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 55.

15 69. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 56.

70. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 57.

20 71. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 58.

72. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 59.

25 73. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 60.

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74. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 61.

5 75. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 62.

76. An isolated nucleic acid which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of the isolated nucleic acid, or a variant thereof, of claim 63.

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